Claims

1. An effective and economical method of processing clinical samples useful for simple, rapid, safe, sensitive, and accurate diagnosis of bacterial infections using a composition comprising solution 1 consisting of Universal Sample Processing (USP) solution (composed of Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M), Solution 2 consisting of Sodium phosphate of concentration ranging between 65 to 70 mM of pH ranging between 6.7 to 6.8 (optional can be replaced with sterile water), and Solution 3 consisting of Tween 80 of concentration ranging between 0.03 to 0.08%, and Solution A comprising Chelex 100 suspension of concentration ranging between 8-12%, Solution B consisting of Triton X-100 of concentration ranging between 0.02-0.04%, and Solution C comprising Tween 20 of concentration ranging between 0.2-0.4% for isolating DNA (optionally, Solution 3 alone or Solution 3 with 0.03-0.1 % Triton X 100 can be used for isolating DNA from high bacillary load or low junk containing samples in place of solutions A, B and C), said method comprising steps of:
    a. obtaining the clinical sample,
    b. mixing 1.5 to 2 volumes of solution 1 to the sample,
    c. homogenizing the mixture while avoiding frothing,
    d. adding Solution 2 or sterile water to the homogenate followed by centrifugation to obtain pellet,
    e. washing the pellet with solution 1, optionally depending upon the decrease of the pellet size,
    f. washing the solution 1-washed pellet with water, and
    g. resuspending the water-washed pellet in solution 3 to obtain processed sample for diagnosis.
2. A method as claimed in claim 1, wherein the processed sample can be used in the form of smear, culture, or Polymerase chain reaction (PCR) starting material, using PCR amplifiable mycobacterial DNA, and RNA.

3. A method as claimed in claim 1, wherein said Universal Sample Processing (USP) solution comprises Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M.

4. A method as claimed in claim 1 where the processed sample can be used for smear microscopy of mycobacteria including *M. tuberculosis* and Gram-positive organisms like *Staphylococcus sp.*

5. A method as claimed in claim 1, wherein homogenizing for time duration ranging between 20-120 seconds.

6. A method as claimed in claim 3, wherein Guanidinium hydrochloride of solution 1 lyses eukaryotic and Gram negative cells, denatures proteins, liquefies sample, and inactivates endogenous enzymes.

7. A method as claimed in claim 1, wherein the processing is completed in a total time duration ranging between 1- 2 hours.

8. A method as claimed in claim 1, wherein said method yields inhibitor-free mycobacterial DNA for PCR based diagnostics of mycobacterial diseases including TB and leprosy.

9. A method as claimed in claim 1, wherein Universal Sample Processing (USP) solution for processing only culture and smear samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 4M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.1M.

10. A method as claimed in claim 1, wherein said Universal Sample Processing (USP) solution for processing culture, smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 5 M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.2 M.

11. A method as claimed in claim 1, wherein said Universal Sample Processing (USP) solution for processing only smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.2 M.

12. A method as claimed in claim 1, wherein Guanidinium Hydrochloride (GuHCl), Sarcosyl, and beta-mercaptoethanol of USP act in a synergistic manner to perform optimal processing of all kinds of clinical samples.

13. A method as claimed in claim 12, wherein presence of beta-mercaptoethanol and Sarcosyl is suitable for optimal processing of mucoid sputum samples.

14. A method as claimed in claim 12, wherein presence of GuHCl is absolutely necessary along with beta-mercaptoethanol and Sarcosyl for optimal processing of mucopurulent sputum samples.

15. A method as claimed in claim 12, wherein GuHCl acts as the principal inhibitor removal component in case of specimens containing blood, by denaturing hemoglobin and removing it from the specimen.

16. A method as claimed in claim 12, wherein GuHCl acts as the principal decontaminating agent of clinical specimens.

17. A method as claimed in claim 1, wherein PCR-amplifiable mycobacterial DNA can be obtained through simple lysis by boiling in presence of Solution 3 or by adding 0.03-0.1 % Triton X 100 without using Solution A, B and C in case of high bacillary load and/or lesser amount of junk containing samples.

18. A method as claimed in claim 1, wherein the processed sample is free of contaminating organisms, proteins, enzymes, and interfering substances.

19. A method as claimed in claim 2, wherein said method under smear microscopy can detect about 300-400 bacilli/ml of the sample.

20. A method as claimed in claim 2, wherein said method has about 30 folds enhancement in sensitivity over the conventional direct smear microscopy method.

21. A method as claimed in claim 2, wherein said method in a smear shows sensitivity ranging between 97-99%.

22. A method as claimed in claim 2, wherein said method in a smear shows specificity ranging between 83-92%.

23. A method as claimed in claim 2, wherein said method shows enhancement in sensitivity by about 18% over CDC method of smear microscopy.

24. A method as claimed in claim 2, wherein said method shows enhancement in sensitivity by about 30% over direct smear method.

25. A method as claimed in claim 2, wherein said method in smears shows positive predictive value ranging between 80-96%.

26. A method as claimed in claim 2, wherein said method in smears shows negative predictive value ranging between 91 -99 %.

27. A method as claimed in claim 2, wherein said method in smears shows diagnostic accuracy of about 91%.

28. A method as claimed in claim 2, wherein said method reduces counterstaining background enabling better viewing of the slides, enhances the gradation of slides, and reduces time and labour for reading a slide.

29. A method as claimed in claim 2, wherein said method carries out decontamination of samples more efficiently as compared to the CDC method.

30. A method as claimed in claim 2, wherein said method in a culture leads to a lag of about 1 week in appearance of the microbial colonies as compared to untreated microbes.

31. A method as claimed in claim 2, wherein said method in a culture shows sensitivity of about 50%.

32. A method as claimed in claim 2, wherein said method is more suitable than CDC method in tropical areas.

33. A method as claimed in claim 2, wherein said method in culture runs at a neutral pH.

34. A method as claimed in claim 2, wherein said method in PCR shows no inhibition of PCR assay with any type of clinical specimens tested.

35. A method as claimed in claim 2, wherein said method in PCR shows sensitivity ranging between 96.5-100%.

36. A method as claimed in claim 2, wherein said method in PCR shows specificity ranging between 71-100%.

37. A method as claimed in claim 2, wherein said method in PCR using said primers shows positive predictive value ranging between 82-100%.

38. A method as claimed in claim 2, wherein said method in PCR using said primers shows negative predictive value of 94-100%.

39. A method as claimed in claim 2, wherein said method in PCR using said primers shows diagnostic accuracy of about 90-100%.

40. A method as claimed in claim 1, wherein samples can be obtained from sources comprising all types of sputum and other body fluids comprising FNAC, pus, pleural fluid, pericardial fluid, joint aspirate, peritoneal fluids, cerebrospinal fluids, endometrial aspirate, synovial fluid, gastric aspirate, endotracheal aspirate, urine, transtracheal aspirate, bronchoalveolar lavage, laryngeal swab and nasopharyngeal swab; body tissues comprising blood, pleural tissue, bone marrow and biopsy; solid organs comprising lymph node, bone, skin, and bovine samples comprising lymph gland, milk, and blood.

41. A method as claimed in claimed 1, wherein samples stored at about -20^0C for upto 2 months can be processed for PCR, smear-microscopy and culture.

42. A method as claimed in claim 2, wherein said method in PCR can be used for both DNA, and RNA.

43. A method as claimed in claim 1, wherein said composition shows mucolytic, decontaminating, protein denaturant, chaotropic, liquefying, tissue softening/digesting, and mycobacteria-releasing action.

44. A method as claimed in claim 2, wherein said method in smear enables more bacilli to be smeared on the slide thereby increases both sensitivity, and efficiency.

45. A method as claimed in claim 2, wherein said method in smear generally converts slides that are graded as 1+ or scanty by the direct method to 2+/3+ or 2+/1+ respectively.

46. A method as claimed in claim 1, wherein said method helps process sputum samples lacking purulence or containing nasopharyngeal discharge and/or saliva and/or blood.

47. A method as claimed in claim 1, wherein said method yields high quality smears from tissue biopsy samples suitable for very sensitive AFB smear microscopy.

48. An effective and economical method of processing clinical samples useful for simple, rapid, safe, sensitive, and accurate diagnosis of Mycobacterial infections especially *tuberculosis* caused by *Mycobacterium tuberculosis* using a

composition comprising solution 1 consisting of Universal Sample Processing (USP) solution (composed of Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M), Solution 2 consisting of Sodium phosphate of concentration ranging between 65 to 70 mM of pH ranging between 6.7 to 6.8 (optional can be replaced with sterile water), and Solution 3 consisting of Tween 80 of concentration ranging between 0.03 to 0.08%, and Solution A comprising Chelex 100 suspension of concentration ranging between 8-12%, Solution B consisting of Triton X-100 of concentration ranging between 0.02-0.04%, and Solution C comprising Tween 20 of concentration ranging between 0.2-0.4% for isolating DNA (optionally, Solution 3 alone or Solution 3 with 0.03-0.1 % Triton X 100 can be used for isolating DNA from high bacillary load or low junk containing samples in place of solutions A, B and C), said method comprising steps of:

a. obtaining the clinical sample,
b. mixing 1.5 to 2 volumes of solution 1 to the sample,
c. homogenizing the mixture while avoiding frothing,
d. adding Solution 2 to the homogenate to obtain pellet, optionally solution 2 can be replaced with sterile water,
e. washing the pellet with solution 1,
f. washing the solution 1-washed pellet with water, and
g. resuspending the water-washed pellet in solution 3 to obtain processed sample for diagnosis.

49. A method as claimed in claim 47, wherein the processed sample can be used in the form of smear, culture, or Polymerase chain reaction (PCR) starting material using PCR amplifiable mycobacterial DNA, and RNA.

50. A method as claimed in claim 47, wherein the processed sample can be used in the form of smear, culture, or Polymerase chain reaction (PCR) starting material, wherein the PCR amplifiable mycobacterial DNA can be obtained by simple lysis by boiling.

51. A method as claimed in claim 47, wherein said Universal Sample Processing (USP) solution comprises Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M.

52. A method as claimed in claim 47, wherein the method maintains viability of the living samples.

53. A method as claimed in claim 47, wherein homogenizing for time duration ranging between 20-120 seconds.

54. A method as claimed in claim 47, wherein Guanidinium hydrochloride of solution 1 lyses eukaryotic and Gram negative cells, denatures proteins, liquefies sample, and inactivates endogenous enzymes.

55. A method as claimed inn claim 47, wherein the processing is completed in a total time duration ranging between 1- 2 hours.

56. A method as claimed in claim 47, wherein said method yields inhibitor-free mycobacterial DNA for PCR based diagnostics.

57. A method as claimed in claim 48, wherein Universal Sample Processing (USP) solution for processing culture and smear samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 4M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.1M.

58. A method as claimed in claim 48, wherein said Universal Sample Processing (USP) solution for processing culture, smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 5 M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.2 M.

59. A method as claimed in claim 48, wherein said Universal Sample Processing (USP) solution for processing smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 6M, Tris-Cl of concentration

ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.2 M.

60. A method as claimed in claim 48, wherein the composition comprises solution 1 consisting of Universal Sample Processing (USP) solution, Solution 2 consisting of Sodium phosphate of concentration ranging between 65 to 70 mM of pH ranging between 6.7 to 6.8, Solution 3 consisting of Tween 80 of concentration ranging between 0.03 to 0.08%, and optionally, Solution A comprising Chelex 100 suspension of concentration ranging between 8-12%, and/or Solution B consisting of Triton X-100 of concentration ranging between 0.02-0.04%, and/or Tween 20 of concentration ranging between 0.2-0.4%

61. A method as claimed in claim 48, wherein the use of Solution A, Solution B, and Solution C can be restricted to isolating DNA from low bacillary load samples only.

62. A method as claimed in claim 48, wherein Guanidinium Hydrochloride (GuHCl), Sarcosyl, and beta-mercaptoethanol of USP act in a synergistic manner to perform optimal processing of all kinds of clinical samples.

63. A method as claimed in claim 48, wherein presence of beta-mercaptoethanol and Sarcosyl is suitable for optimal processing of mucoid sputum samples.

64. A method as claimed in claim 48, wherein presence of GuHCl is absolutely necessary along with beta-mercaptoethanol and Sarcosyl for optimal processing of mucopurulent sputum samples.

65. A method as claimed in claim 48, wherein GuHCl acts as the principal inhibitor removal component in case of specimens containing blood, by denaturing hemoglobin and removing it from the specimen.

66. A method as claimed in claim 48,wherein GuHCl acts as the principal decontaminating agent of clinical specimens.

67. A method as claimed in claim 48, wherein PCR-amplifiable mycobacterial DNA can be obtained through simple lysis by boiling in presence of 0.04-0.06% Tween 80 or by adding 0.03-0.1 % Triton X 100 without using Solution A, B and C in case of high bacillary load and/or lesser amount of junk containing samples.

68. A method as claimed in claim 47, wherein the processed sample is free of contaminating organisms, proteins, enzymes, and interfering substances.

69. A method as claimed in claim 48, wherein said method under smear microscopy can detect 300-400 bacilli/ml of the sample.

70. A method as claimed in claim 48 wherein smear microscopy can detect fewer than 300-400 bacilli/ml of the sample by using more than 10% of the processed sample in smear preparation.

71. A method as claimed in claim 48, wherein said method has about 30 folds enhancement in sensitivity over the conventional direct smear microscopy method.

72. A method as claimed in claim 48, wherein said method in a smear shows sensitivity ranging between 97-99%.

73. A method as claimed in claim 48, wherein said method in a smear shows specificity ranging between 83-92%.

74. A method as claimed in claim 48, wherein said method shows enhancement in sensitivity by about 18% over CDC method of smear microscopy.

75. A method as claimed in claim 48, wherein said method shows enhancement in sensitivity by about 30% over direct smear method.

76. A method as claimed in claim 48, wherein said method in smears shows positive predictive value ranging between 80-96%.

77. A method as claimed in claim 48, wherein said method in smears shows negative predictive value ranging between 91 -99 %.

78. A method as claimed in claim 48, wherein said method in smears shows diagnostic accuracy of about 91%.

79. A method as claimed in claim 48, wherein said method reduces counterstaining background enabling better viewing of the slides, enhances the gradation of slides, and reduces time and labour for reading a slide.

80. A method as claimed in claim 48, wherein said method carries out decontamination of samples more efficiently as compared to the CDC method.

81. A method as claimed in claim 48, wherein said method in a culture shows twice the viability of the microbes as compared to CDC method.

82. A method as claimed in claim 48, wherein said method in a culture leads to a lag of about 1 week in appearance of the microbial colonies as compared to untreated microbes.

83. A method as claimed in claim 48, wherein said method in a culture shows sensitivity of about 50%.

84. A method as claimed in claim 48, wherein said method is more suitable in than for CDC method in tropical areas.

85. A method as claimed in claim 48, wherein said method in culture runs at a neutral pH.

86. A method as claimed in claim 48, wherein said method in PCR shows no inhibition of PCR assay.

87. A method as claimed in claim 48, wherein said method in PCR shows two sets of primers namely, devRf2 & devRr2, and devRf3, & devRr3 of gene devR of microbe Mycobacterium tuberculosis.

88. A method as claimed in claim 79, wherein the primers devRf2, and devRr2 amplify a 308bp fragment of gene devR of microbe Mycobacterium tuberculosis.

89. A method as claimed in claim 79, wherein the primers devRf3, and devRr3 amplify a 164 bp fragment of gene *devR* of microbe *Mycobacterium tuberculosis.*

90. A method as claimed in claim 79, wherein said method in PCR using primers devRf2 and devRr2 shows 2-4 folds increase in sensitivity as compared to devRf and devRr.

91. A method as claimed in claim 79, wherein said method in PCR using primers devRf3 and devRr3 shows at least 10 folds increase in sensitivity as compared to primers devRf and devRr.

92. A method as claimed in claim 48, wherein samples can be obtained from sources comprising all types of sputum and other body fluids comprising FNAC, pus, pleural fluid, pericardial fluid, joint aspirate, peritoneal fluids, cerebrospinal fluids, endometrial aspirate, synovial fluid, gastric aspirate, endotracheal aspirate, urine, transtracheal aspirate, bronchoalveolar lavage, laryngeal swab and nasopharyngeal swab; body tissues comprising blood, pleural tissue, bone marrow and biopsy; solid organs comprising lymph node, bone, skin, and bovine samples comprising lymph gland, milk, and blood.

93. A method as claimed in claimed 48, wherein samples stored at about $-20^{0}C$ for upto 2 months can be processed for PCR, smear-microscopy and culture.

94. A method as claimed in claim 48, wherein said method in PCR can be used for both DNA, and RNA.

95. A method as claimed in claim 48, wherein said composition shows mucolytic, decontaminating, protein denaturant, chaotropic, liquefying, tissue softening/digesting, and mycobacteria-releasing action.

96. A method as claimed in claim 48, wherein said method in smear enables more bacilli to be smeared on the slide thereby increasing the sensitivity and the efficiency.

97. A method as claimed in claim 48, wherein said method in smear generally converts slides that are graded as 1+ or scanty by the direct method to 2+/3+ or 2+/1+ respectively.

98. A method as claimed in claim 48, wherein samples lacking purulence or containing nasopharyngeal discharge or saliva can be processed.

99. A method as claimed in claim 48, wherein said method yields high quality smears from tissue biopsy samples suitable for very sensitive AFB smear microscopy.

100. A method as claimed in claim 48, wherein the said method shows no adverse effect on the acid-fast properties, viability, and integrity of the *Mycobacterium tuberculosis.*

101. A method as claimed in claim 47, wherein said method is suitable for diagnosis of pulmonary as well as extrapulmonary tuberculosis with equal efficacy.

102. A method as claimed in claim 47, wherein said method is compatible with culturing mycobacterium from clinical specimens in both solid and liquid media.

103. A method as claimed in claim 48, wherein the said method can detect samples as positive which have been detected negative by direct and CDC methods of smear microscopy.

104. A Kit useful in processing clinical samples for simple, rapid, safe, sensitive, and accurate diagnosis of microbial disease conditions, said kit comprising solution 1 consisting of Universal Sample Processing (USP) solution (composed of Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M) , Solution 2 consisting of Sodium phosphate of concentration ranging between 65 to 70 mM of pH ranging between 6.7 to 6.8 (optionally can be replaced with water), Solution 3 consisting of Tween 80 of concentration ranging between 0.03 to 0.08%, Solution A comprising Chelex 100 suspension of concentration ranging between 8-12%, Solution B consisting of Triton X-100 of concentration ranging between 0.02-0.04%, and Solution C consisting of Tween 20 of concentration ranging between 0.2-

0.4%, optionally two sets of primers with devRf2 and devRr2 of SEQ ID No. 1 and SEQ ID No. 2 respectively, and primers devRf3, and devRr3 of SEQ ID No. 3, and SEQ ID No. 4 respectively.

105. A kit as claimed in claim 104, wherein said kit is useful in processing clinical samples for detecting bacterial infections.

106. A kit as claimed in claim 104, wherein said kit is useful in processing clinical samples for detecting tuberculosis.

107. A kit as claimed in claim 104, wherein said Universal Sample Processing (USP) solution comprises Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6 M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M.

108. A kit as claimed in claim 104, wherein Universal Sample Processing (USP) solution for processing culture and smear samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 4M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.1M.

109. A kit as claimed in claim 104, wherein said Universal Sample Processing (USP) solution for processing culture, smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 5M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30 mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.2 M.

110. A kit as claimed in claim 104, wherein said Universal Sample Processing (USP) solution for processing smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.2 M.

111. A kit as claimed in claim 104, wherein the composition comprises solution 1 consisting of Universal Sample Processing (USP) solution, Solution 2 consisting of Sodium phosphate of concentration ranging between 65 to 70 mM of pH ranging between 6.7 to 6.8, Solution 3 consisting of Tween 80 of concentration ranging between 0.03 to 0.08%, and optionally, Solution A comprising Chelex 100 suspension of concentration ranging between 8-12%, and Solution B consisting of Triton X-100 of concentration ranging between 0.02-0.04%, and Solution C consisting of Tween 20 of concentration ranging between 0.2-0.4%.

112. A set of primers devRf2 and devRr2 of SEQ ID No. 1 and SEQ ID No. 2 respectively.

113. A set of primers devRf3, and devRr3 of SEQ ID No. 3, and SEQ ID No. 4 respectively.

114. A method of using primers of SEQ ID NO. 1, and 2 or SEQ ID No. 3 and 4 of gene *devR* for screening patients of tuberculosis said method comprising steps of conducting Polymerase Chain Reaction (PCR) using DNA or RNA of the processed sample of the subject, identifying the subjects suffering from tuberculosis.

115. A processed clinical sample obtained by processing a clinical specimen using method of claim 1.

116. A processed sample as claimed in claim 112, said clinical specimen comprising all types of sputum and other body fluids comprising FNAC, pus, pleural fluid, pericardial fluid, joint aspirate, peritoneal fluids, cerebrospinal fluids, endometrial aspirate, synovial fluid, gastric aspirate, endotracheal aspirate, urine, transtracheal aspirate, bronchoalveolar lavage, laryngeal swab and nasopharyngeal swab; body tissues comprising blood, pleural tissue, bone marrow and biopsy; solid organs comprising lymph node, bone, skin, and bovine samples comprising lymph gland, milk, and blood.

AMENDED CLAIMS

[received by the International Bureau on 09 July 2004 (09.07.2004); original claims 1-116 replaced by new claims 1-114 (14 pages)]

+ STATEMENT

Claims

1. An effective and economical method of processing clinical samples useful for simple, rapid, safe, sensitive, and accurate diagnosis of bacterial infections using a composition comprising solution 1 consisting of Universal Sample Processing (USP) solution (composed of Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M), Solution 2 consisting of Sodium phosphate of concentration ranging between 65 to 70 mM of pH ranging between 6.7 to 6.8 (optional can be replaced with sterile water), and Solution 3, consisting of Tween 80 of concentration ranging between 0.03 to 0.08%, and Solution A comprising Chelex 100 suspension of concentration ranging between 8-12%, Solution B consisting of Triton X-100 of concentration ranging between 0.02-0.04%, and Solution C comprising Tween 20 of concentration ranging between 0.2-0.4% for isolating DNA (optionally, Solution 3 alone or Solution 3 with 0.03-0.1 % Triton X 100 can be used for isolating DNA from high bacillary load or low junk containing samples in place of solutions A, B and C), said method comprising steps of:
    a. obtaining the clinical sample,
    b. mixing 1.5 to 2 volumes of solution 1 to the sample,
    c. homogenizing the mixture while avoiding frothing,
    d. adding Solution 2 or sterile water to the homogenate followed by centrifugation to obtain pellet,
    e. washing the pellet with solution 1, optionally depending upon the decrease of the pellet size,
    f. washing the solution 1-washed pellet with water, and
    g. resuspending the water-washed pellet in solution 3 to obtain processed sample for diagnosis.

2. A method as claimed in claim 1, wherein the processed sample can be used in the form of smear, culture, or Polymerase chain reaction (PCR) starting material, using PCR amplifiable mycobacterial DNA, and RNA.
3. A method as claimed in claim 1, wherein said Universal Sample Processing (USP) solution comprises Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M.
4. A method as claimed in claim 1 where the processed sample can be used for smear microscopy of mycobacteria including *M. tuberculosis* and Gram-positive organisms like *Staphylococcus sp.*
5. A method as claimed in claim 1, wherein homogenizing for time duration ranging between 20-120 seconds.
6. A method as claimed in claim 3, wherein Guanidinium hydrochloride of solution 1 lyses eukaryotic and Gram negative cells, denatures proteins, liquefies sample, and inactivates endogenous enzymes.
7. A method as claimed in claim 1, wherein the processing is completed in a total time duration ranging between 1- 2 hours.
8. A method as claimed in claim 1, wherein said method yields inhibitor-free mycobacterial DNA for PCR based diagnostics of mycobacterial diseases including TB and leprosy.
9. A method as claimed in claim 1, wherein Universal Sample Processing (USP) solution for processing only culture and smear samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 4M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.1M.
10. A method as claimed in claim 1, wherein said Universal Sample Processing (USP) solution for processing culture, smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 5 M, Tris-Cl of

concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.2 M.

11. A method as claimed in claim 1, wherein said Universal Sample Processing (USP) solution for processing only smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.2 M.
12. A method as claimed in claim 1, wherein Guanidinium Hydrochloride (GuHCl), Sarcosyl, and beta-mercaptoethanol of USP act in a synergistic manner to perform optimal processing of all kinds of clinical samples.
13. A method as claimed in claim 12, wherein presence of beta-mercaptoethanol and Sarcosyl is suitable for optimal processing of mucoid sputum samples.
14. A method as claimed in claim 12, wherein presence of GuHCl is absolutely necessary along with beta-mercaptoethanol and Sarcosyl for optimal processing of mucopurulent sputum samples.
15. A method as claimed in claim 12, wherein GuHCl acts as the principal inhibitor removal component in case of specimens containing blood, by denaturing hemoglobin and removing it from the specimen.
16. A method as claimed in claim 12, wherein GuHCl acts as the principal decontaminating agent of clinical specimens.
17. A method as claimed in claim 1, wherein PCR-amplifiable mycobacterial DNA can be obtained through simple lysis by boiling in presence of Solution 3 or by adding 0.03-0.1 % Triton X 100 without using Solution A, B and C in case of high bacillary load and/or lesser amount of junk containing samples.
18. A method as claimed in claim 1, wherein the processed sample is free of contaminating organisms, proteins, enzymes, and interfering substances.
19. A method as claimed in claim 2, wherein said method under smear microscopy can detect about 300-400 bacilli/ml of the sample.

20. A method as claimed in claim 2, wherein said method has about 30 folds enhancement in sensitivity over the conventional direct smear microscopy method.
21. A method as claimed in claim 2, wherein said method in a smear shows sensitivity ranging between 97-99%.
22. A method as claimed in claim 2, wherein said method in a smear shows specificity ranging between 83-92%.
23. A method as claimed in claim 2, wherein said method shows enhancement in sensitivity by about 18% over CDC method of smear microscopy.
24. A method as claimed in claim 2, wherein said method shows enhancement in sensitivity by about 30% over direct smear method.
25. A method as claimed in claim 2, wherein said method in smears shows positive predictive value ranging between 80-96%.
26. A method as claimed in claim 2, wherein said method in smears shows negative predictive value ranging between 91 -99 %.
27. A method as claimed in claim 2, wherein said method in smears shows diagnostic accuracy of about 91%.
28. A method as claimed in claim 2, wherein said method reduces counterstaining background enabling better viewing of the slides, enhances the gradation of slides, and reduces time and labour for reading a slide.
29. A method as claimed in claim 2, wherein said method carries out decontamination of samples more efficiently as compared to the CDC method.
30. A method as claimed in claim 2, wherein said method in a culture leads to a lag of about 1 week in appearance of the microbial colonies as compared to untreated microbes.
31. A method as claimed in claim 2, wherein said method in a culture shows sensitivity of about 50%.
32. A method as claimed in claim 2, wherein said method is more suitable than CDC method in tropical areas.
33. A method as claimed in claim 2, wherein said method in culture runs at a neutral pH.

34. A method as claimed in claim 2, wherein said method in PCR shows no inhibition of PCR assay with any type of clinical specimens tested.

35. A method as claimed in claim 2, wherein said method in PCR shows sensitivity ranging between 96.5-100%.

36. A method as claimed in claim 2, wherein said method in PCR shows specificity ranging between 71-100%.

37. A method as claimed in claim 2, wherein said method in PCR using said primers shows positiye predictive value ranging between 82-100%.

38. A method as claimed in claim 2, wherein said method in PCR using said primers shows negative predictive value of 94-100%.

39. A method as claimed in claim 2, wherein said method in PCR using said primers shows diagnostic accuracy of about 90-100%.

40. A method as claimed in claim 1, wherein samples can be obtained from sources comprising all types of sputum and other body fluids comprising FNAC, pus, pleural fluid, pericardial fluid, joint aspirate, peritoneal fluids, cerebrospinal fluids, endometrial aspirate, synovial fluid, gastric aspirate, endotracheal aspirate, urine, transtracheal aspirate, bronchoalveolar lavage, laryngeal swab and nasopharyngeal swab; body tissues comprising blood, pleural tissue, bone marrow and biopsy; solid organs comprising lymph node, bone, skin, and bovine samples comprising lymph gland, milk, and blood.

41. A method as claimed in claimed 1, wherein samples stored at about -20^0C for upto 2 months can be processed for PCR, smear-microscopy and culture.

42. A method as claimed in claim 2, wherein said method in PCR can be used for both DNA, and RNA.

43. A method as claimed in claim 1, wherein said composition shows mucolytic, decontaminating, protein denaturant, chaotropic, liquefying, tissue softening/digesting, and mycobacteria-releasing action.

44. A method as claimed in claim 2, wherein said method in smear enables more bacilli to be smeared on the slide thereby increases both sensitivity, and efficiency.

45. A method as claimed in claim 2, wherein said method in smear generally converts slides that are graded as 1+ or scanty by the direct method to 2+/3+ or 2+/1+ respectively.

46. A method as claimed in claim 1, wherein said method helps process sputum samples lacking purulence or containing nasopharyngeal discharge and/or saliva and/or blood.

47. A method as claimed in claim 1, wherein said method yields high quality smears from tissue biopsy samples suitable for very sensitive AFB smear microscopy.

48. An effective and economical method of processing clinical samples useful for simple, rapid, safe, sensitive, and accurate diagnosis of Mycobacterial infections especially *tuberculosis* caused by *Mycobacterium tuberculosis* using a composition comprising solution 1 consisting of Universal Sample Processing (USP) solution (composed of Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M), Solution 2 consisting of Sodium phosphate of concentration ranging between 65 to 70 mM of pH ranging between 6.7 to 6.8 (optional can be replaced with sterile water), and Solution 3 consisting of Tween 80 of concentration ranging between 0.03 to 0.08%, and Solution A comprising Chelex 100 suspension of concentration ranging between 8-12%, Solution B consisting of Triton X-100 of concentration ranging between 0.02-0.04%, and Solution C comprising Tween 20 of concentration ranging between 0.2-0.4% for isolating DNA (optionally, Solution 3 alone or Solution 3 with 0.03-0.1 % Triton X 100 can be used for isolating DNA from high bacillary load or low junk containing samples in place of solutions A, B and C), said method comprising steps of:

a. obtaining the clinical sample,
b. mixing 1.5 to 2 volumes of solution 1 to the sample,
c. homogenizing the mixture while avoiding frothing,

d. adding Solution 2 to the homogenate to obtain pellet, optionally solution 2 can be replaced with sterile water,
e. washing the pellet with solution 1,
f. washing the solution 1-washed pellet with water, and
g. resuspending the water-washed pellet in solution 3 to obtain processed sample for diagnosis.

49. A method as claimed in claim 47, wherein the processed sample can be used in the form of smear, culture, or Polymerase chain reaction (PCR) starting material using PCR amplifiable mycobacterial DNA, and RNA.

50. A method as claimed in claim 47, wherein the processed sample can be used in the form of smear, culture, or Polymerase chain reaction (PCR) starting material, wherein the PCR amplifiable mycobacterial DNA can be obtained by simple lysis by boiling.

51. A method as claimed in claim 47, wherein said Universal Sample Processing (USP) solution comprises Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M.

52. A method as claimed in claim 47, wherein the method maintains viability of the living samples.

53. A method as claimed in claim 47, wherein homogenizing for time duration ranging between 20-120 seconds.

54. A method as claimed in claim 47, wherein Guanidinium hydrochloride of solution 1 lyses eukaryotic and Gram negative cells, denatures proteins, liquefies sample, and inactivates endogenous enzymes.

55. A method as claimed inn claim 47, wherein the processing is completed in a total time duration ranging between 1- 2 hours.

56. A method as claimed in claim 47, wherein said method yields inhibitor-free mycobacterial DNA for PCR based diagnostics.

57. A method as claimed in claim 48, wherein Universal Sample Processing (USP) solution for processing culture and smear samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 4M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.1M.

58. A method as claimed in claim 48, wherein said Universal Sample Processing (USP) solution for processing culture, smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 5 M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.2 M.

59. A method as claimed in claim 48, wherein said Universal Sample Processing (USP) solution for processing smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.2 M.

60. A method as claimed in claim 48, wherein the composition comprises solution 1 consisting of Universal Sample Processing (USP) solution, Solution 2 consisting of Sodium phosphate of concentration ranging between 65 to 70 mM of pH ranging between 6.7 to 6.8, Solution 3 consisting of Tween 80 of concentration ranging between 0.03 to 0.08%, and optionally, Solution A comprising Chelex 100 suspension of concentration ranging between 8-12%, and/or Solution B consisting of Triton X-100 of concentration ranging between 0.02-0.04%, and/or Tween 20 of concentration ranging between 0.2-0.4%

61. A method as claimed in claim 48, wherein the use of Solution A, Solution B, and Solution C can be restricted to isolating DNA from low bacillary load samples only.

62. A method as claimed in claim 48, wherein Guanidinium Hydrochloride (GuHCl), Sarcosyl, and beta-mercaptoethanol of USP act in a synergistic manner to perform optimal processing of all kinds of clinical samples.
63. A method as claimed in claim 48, wherein presence of beta-mercaptoethanol and Sarcosyl is suitable for optimal processing of mucoid sputum samples.
64. A method as claimed in claim 48, wherein presence of GuHCl is absolutely necessary along with beta-mercaptoethanol and Sarcosyl for optimal processing of mucopurulent sputum samples.
65. A method as claimed in claim 48, wherein GuHCl acts as the principal inhibitor removal component in case of specimens containing blood, by denaturing hemoglobin and removing it from the specimen.
66. A method as claimed in claim 48,wherein GuHCl acts as the principal decontaminating agent of clinical specimens.
67. A method as claimed in claim 48, wherein PCR-amplifiable mycobacterial DNA can be obtained through simple lysis by boiling in presence of 0.04-0.06% Tween 80 or by adding 0.03-0.1 % Triton X 100 without using Solution A, B and C in case of high bacillary load and/or lesser amount of junk containing samples.
68. A method as claimed in claim 47, wherein the processed sample is free of contaminating organisms, proteins, enzymes, and interfering substances.
69. A method as claimed in claim 48, wherein said method under smear microscopy can detect 300-400 bacilli/ml of the sample.
70. A method as claimed in claim 48 wherein smear microscopy can detect fewer than 300-400 bacilli/ml of the sample by using more than 10% of the processed sample in smear preparation.
71. A method as claimed in claim 48, wherein said method has about 30 folds enhancement in sensitivity over the conventional direct smear microscopy method.
72. A method as claimed in claim 48, wherein said method in a smear shows sensitivity ranging between 97-99%.
73. A method as claimed in claim 48, wherein said method in a smear shows specificity ranging between 83-92%.

74. A method as claimed in claim 48, wherein said method shows enhancement in sensitivity by about 18% over CDC method of smear microscopy.
75. A method as claimed in claim 48, wherein said method shows enhancement in sensitivity by about 30% over direct smear method.
76. A method as claimed in claim 48, wherein said method in smears shows positive predictive value ranging between 80-96%.
77. A method as claimed in claim 48, wherein said method in smears shows negative predictive value ranging between 91 -99 %.
78. A method as claimed in claim 48, wherein said method in smears shows diagnostic accuracy of about 91%.
79. A method as claimed in claim 48, wherein said method reduces counterstaining background enabling better viewing of the slides, enhances the gradation of slides, and reduces time and labour for reading a slide.
80. A method as claimed in claim 48, wherein said method carries out decontamination of samples more efficiently as compared to the CDC method.
81. A method as claimed in claim 48, wherein said method in a culture shows twice the viability of the microbes as compared to CDC method.
82. A method as claimed in claim 48, wherein said method in a culture leads to a lag of about 1 week in appearance of the microbial colonies as compared to untreated microbes.
83. A method as claimed in claim 48, wherein said method in a culture shows sensitivity of about 50%.
84. A method as claimed in claim 48, wherein said method is more suitable in than for CDC method in tropical areas.
85. A method as claimed in claim 48, wherein said method in culture runs at a neutral pH.
86. A method as claimed in claim 48, wherein said method in PCR shows no inhibition of PCR assay.
87. A method as claimed in claim 48, wherein said method in PCR shows two sets of primers namely, devRf2 & devRr2, and devRf3, & devRr3 of gene devR of microbe Mycobacterium tuberculosis.

88. A method as claimed in claim 79, wherein the primers devRf2, and devRr2 amplify a 308bp fragment of gene devR of microbe Mycobacterium tuberculosis.

89. A method as claimed in claim 79, wherein the primers devRf3, and devRr3 amplify a 164 bp fragment of gene *devR* of microbe *Mycobacterium tuberculosis*.

90. A method as claimed in claim 79, wherein said method in PCR using primers devRf2 and devRr2 shows 2-4 folds increase in sensitivity as compared to devRf and devRr.

91. A method as claimed in claim 79, wherein said method in PCR using primers devRf3 and devRr3 shows at least 10 folds increase in sensitivity as compared to primers devRf and devRr.

92. A method as claimed in claim 48, wherein samples can be obtained from sources comprising all types of sputum and other body fluids comprising FNAC, pus, pleural fluid, pericardial fluid, joint aspirate, peritoneal fluids, cerebrospinal fluids, endometrial aspirate, synovial fluid, gastric aspirate, endotracheal aspirate, urine, transtracheal aspirate, bronchoalveolar lavage, laryngeal swab and nasopharyngeal swab; body tissues comprising blood, pleural tissue, bone marrow and biopsy; solid organs comprising lymph node, bone, skin, and bovine samples comprising lymph gland, milk, and blood.

93. A method as claimed in claimed 48, wherein samples stored at about -20^0C for upto 2 months can be processed for PCR, smear-microscopy and culture.

94. A method as claimed in claim 48, wherein said method in PCR can be used for both DNA, and RNA.

95. A method as claimed in claim 48, wherein said composition shows mucolytic, decontaminating, protein denaturant, chaotropic, liquefying, tissue softening/digesting, and mycobacteria-releasing action.

96. A method as claimed in claim 48, wherein said method in smear enables more bacilli to be smeared on the slide thereby increasing the sensitivity and the efficiency.

97. A method as claimed in claim 48, wherein said method in smear generally converts slides that are graded as 1+ or scanty by the direct method to 2+/3+ or 2+/1+ respectively.

98. A method as claimed in claim 48, wherein samples lacking purulence or containing nasopharyngeal discharge or saliva can be processed.

99. A method as claimed in claim 48, wherein said method yields high quality smears from tissue biopsy samples suitable for very sensitive AFB smear microscopy.

100. A method as claimed in claim 48, wherein the said method shows no adverse effect on the acid-fast properties, viability, and integrity of the *Mycobacterium tuberculosis.*

101. A method as claimed in claim 47, wherein said method is suitable for diagnosis of pulmonary as well as extrapulmonary tuberculosis with equal efficacy.

102. A method as claimed in claim 47, wherein said method is compatible with culturing mycobacterium from clinical specimens in both solid and liquid media.

103. A method as claimed in claim 48, wherein the said method can detect samples as positive which have been detected negative by direct and CDC methods of smear microscopy.

104. A Kit useful in processing clinical samples for simple, rapid, safe, sensitive, and accurate diagnosis of microbial disease conditions, said kit comprising solution 1 consisting of Universal Sample Processing (USP) solution (composed of Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M) , Solution 2 consisting of Sodium phosphate of concentration ranging between 65 to 70 mM of pH ranging between 6.7 to 6.8 (optionally can be replaced with water), Solution 3 consisting of Tween 80 of concentration ranging between 0.03 to 0.08%, Solution A comprising Chelex 100 suspension of concentration ranging between 8-12%, Solution B consisting of Triton X-100 of concentration ranging between 0.02-0.04%, and Solution C consisting of Tween 20 of concentration ranging between 0.2-0.4%, optionally two sets of primers with devRf2 and devRr2 of SEQ ID No. 1 and SEQ ID No. 2 respectively, and primers devRf3, and devRr3 of SEQ ID No. 3, and SEQ ID No. 4 respectively.

105. A kit as claimed in claim 104, wherein said kit is useful in processing clinical samples for detecting bacterial infections.

106. A kit as claimed in claim 104, wherein said kit is useful in processing clinical samples for detecting tuberculosis.

107. A kit as claimed in claim 104, wherein said Universal Sample Processing (USP) solution comprises Guanidinium Hydrochloride (GuHCl) of concentration ranging between 3-6 M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.3 M.

108. A kit as claimed in claim 104, wherein Universal Sample Processing (USP) solution for processing culture and smear samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 4M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.1M.

109. A kit as claimed in claim 104, wherein said Universal Sample Processing (USP) solution for processing culture, smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 5M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30 mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration ranging between 0.1-0.2 M.

110. A kit as claimed in claim 104, wherein said Universal Sample Processing (USP) solution for processing smear, and PCR samples comprises Guanidinium Hydrochloride (GuHCl) of concentration of about 6M, Tris-Cl of concentration ranging between 40-60 mM of pH ranging between 7.3- 7.7, EDTA of concentration ranging between 20-30mM, Sarcosyl of concentration ranging between 0.3 – 0.8%, beta-mercaptoethanol of concentration of about 0.2 M.

111. A kit as claimed in claim 104, wherein the composition comprises solution 1 consisting of Universal Sample Processing (USP) solution, Solution 2 consisting of Sodium phosphate of concentration ranging between 65 to 70 mM of pH ranging between 6.7 to 6.8, Solution 3 consisting of Tween 80 of concentration

ranging between 0.03 to 0.08%, and optionally, Solution A comprising Chelex 100 suspension of concentration ranging between 8-12%, and Solution B consisting of Triton X-100 of concentration ranging between 0.02-0.04%, and Solution C consisting of Tween 20 of concentration ranging between 0.2-0.4%.

112. A set of primers devRf2 and devRr2 of SEQ ID No. 1 and SEQ ID No. 2 respectively.

113. A set of primers devRf3, and devRr3 of SEQ ID No. 3, and SEQ ID No. 4 respectively.

114. A method of using primers of SEQ ID NO. 1, and 2 or SEQ ID No. 3 and 4 of gene *devR* for screening patients of tuberculosis said method comprising steps of conducting Polymerase Chain Reaction (PCR) using DNA or RNA of the processed sample of the subject, identifying the subjects suffering from tuberculosis.

STATEMENT UNDER ARTICLE 19(1)

The Applicant has deleted the claims 115 and 116 to overcome the rejections set by the Examiner.

Thus, we respectfully request the Examiner to have a favorable consideration of the response.

Lastly, the Applicant is not keen to pay an additional fee for further searches for the claims 112 to 114.